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PHYSICAL CHEMISTRY

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Abstract

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DESTRUCTION AND FORMATION OF STRUCTURES OF NATURAL PROTEINS DURING THEIR TRANSFER THROUGH NONPOROUS MEMBRANES

The entire diversity of vital phenomena is usually determined by the manifestation of the chemical and physicochemical properties of the substances that form the basis of organisms of living nature and by their interaction with the substances of the surrounding medium in directions corresponding to the specificity of biological processes. However, for an understanding of these processes, the study of structural problems also acquires great importance, i.e., the regularities of the formation and destruction of structures in living organisms, the characterization of types of structures, their morphology and nature. It was therefore of interest to consider the character of the destruction and formation of microstructures of natural proteins by investigating the well-known phenomenon of protein transfer through nonporous (cellophane) membranes during electrodialysis.

For this purpose a high-voltage multicompartment electrodialyzer was used, the advantage of which consists in the fact that, owing to the presence of "electric traps," the dynamic equilibrium in a system with a very small fraction of dispersed substance is constantly shifted toward dissociation into elementary structural units ([1]). The objects of investigation were fibrillar and globular proteins: gelatin, myosin (in the form of finely ground muscle tissue), egg albumin (in the form of denatured chicken-egg white), and edestin. As the principal method of investigation in the present work, the electron-microscopic method was employed, using a JEM-5Y electron microscope at direct magnifications from 15,000 to 85,000×.

Destruction of the structures of natural proteins and their transfer through nonporous membranes during electrodialysis. In studying the processes of electrodialysis of proteins earlier ([2]), it was observed that certain proteins of comparatively high molecular weight are capable of passing through nonporous polymer membranes. This phenomenon also occurred in the electrodialysis of the proteins studied by us in a five-chamber high-voltage electrodialyzer. Globular proteins (edestin, denatured chicken-egg white), as well as one of the fibrillar proteins—myosin—were transferred into the neighboring chamber through the cellophane membrane in insignificant amounts, whereas the removal

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of gelatin, carried out under the indicated conditions, amounted to rather large quantities.

This observation prompted a more detailed study of this phenomenon using gelatin solutions as an example. For this purpose, the influence on the transfer processes of the gelatin concentration (from 1.5 to 10%) and of temperature (from 25 to 50°) was investigated at various voltages on the electrodes of the electrodialyzer (from 0.05 to 2.5 kV). It turned out that increasing the concentration and temperature promotes an increase in the transfer of gelatin through the membrane into the neighboring chamber of the electrodialyzer.

Repeated experiments made it possible to show that, by using the temperature factor, it is possible to achieve transfer of gelatin through the membrane in co-

amounts up to 30–40%. Naturally, the question arose as to the causes of this phenomenon. The transfer of small initial amounts of gelatin and other proteins studied is apparently due to the presence in the proteins of fractions with a comparatively low molecular weight, which, in the process of electrodialysis, are capable of passing through nonporous membranes. To clarify the reasons for the transfer of large amounts of gelatin, special experiments were set up to determine the molecular weights of the initial gelatin and of the gelatin transferred into the neighboring chamber of the electrodialyzer. The average molecular weight of the initial gelatin was 41,350; after electrodialysis it fell to 23,590, while the molecular weights of the gelatin fractions transferred through the membrane were: at $t = 60°$, 23,140, and at $t = 80°$, 22,680. Thus, the transfer of gelatin through the membrane is not associated with a decrease in the molecular weight of gelatin, for if this were so, all the gelatin should have been transferred in the process of electrodialysis through the membrane, since the molecular weights of the gelatin subjected to electrodialysis and not passing through the membrane, and of the gelatin that passed through the membrane, coincide.

Special experiments were then set up to determine the role of the pH of gelatin solutions and the influence on the transfer process of changes in the acid–base equilibrium. It turned out that repeated addition of sulfuric acid did not increase the transfer of gelatin. The situation changed sharply if, in the course of electrodialysis, after complete cessation of protein transfer through the membrane, small amounts of surface-active substances were introduced into the system containing the initial protein. As such substances, both surface-active electrolytes (digexyl ester of the Na salt of sulfosuccinic acid) and surface-active nonelectrolytes (OP-10) were used. Upon addition of several drops of a solution of either surface-active substance, the protein transfer that had ceased was immediately resumed; this applied to the same extent to all fibrillar and globular proteins that we studied. Electrodialysis of gelatin in urea solutions also promoted an increase in the amount of product transferred through the membrane, which was verified in a special series of experiments. In this case, even at reduced temperatures and voltages, the transfer of gelatin reaches, in a comparatively short time, 80% of the initial weight of the gelatin subjected to electrodialysis.

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This remarkable phenomenon sheds light on the causes of protein transfer through nonporous membranes and at the same time compels us to reconsider our usual ideas about the structure of protein molecules. Svedberg [3] had already discovered the phenomenon of reversible dissociation of the protein molecule into separate elements—blocks, connected with one another into a single chain by bonds capable of being easily broken and again restored under definite conditions of action. Such a primary structure of protein molecular formations agrees well with modern experimental data on the study of the process of protein decomposition and the synthesis of complex structures from them by the method of isotopic indicators [4]. Apparently, the process of protein transfer through nonporous membranes during electrodialysis is connected precisely with these features of the complex construction of the primary structures of proteins. In a five-chamber electrodialyzer there occurs continuous decomposition of the primary structures of proteins into separate molecules of small size—blocks—which are easily transferred through the membranes into the side chambers at high speed. An increase in temperature and in the applied voltage facilitates the process of structural decomposition. The action of surface-active substances, which sharply increase the transfer of protein through membranes, may be imagined by analogy with the action of soaps.

The processes of structure formation and the character of secondary structures of proteins transferred through nonporous membranes during electrodialysis. To study the processes of the formation of secondary structural formations

Fig. 1

Scale: 0.25μ

Fig. 2

a –scale: 0.5μ

b –scale: 0.5μ

Fig. 3

a –scale: 0.5μ

b –scale: 0.5μ

Fig. 4

a –scale: 0.5μ

b –scale: 0.5μ

of proteins from their elements that had passed through a nonporous membrane during electrodialysis, direct observation of the structures formed in an electron microscope was used. The application of such a method proved possible when two conditions were met: the high resolving power of the electron microscope

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and prevention of the possibility of formation of thin protein films, which cannot be examined in the electron microscope, since they do not have the contrast necessary for detecting the structural features of the object. The resolving power of the JEM-5Y electron microscope, equal to 8-10 Å, made it possible to fulfill the first condition. The second condition was fulfilled by using solutions of very low concentrations (down to 0.003%) and drying them at temperatures above the gelation point. For gelatin, drying of the solution was carried out at 40°. In this case a jelly does not form, and it is possible to observe the secondary structural formations of the protein that arise after the solution has dried.

Observing the conditions set forth, it was possible to obtain, in electron-microscopic photographs, secondary structural formations of the initial fibrillar proteins—gelatin and myosin—which have a globular character. Fig. 1 shows an image of gelatin globules. Similar globules, differing only in smaller size, are formed by myosin. The phenomenon of globulization of gelatin molecules in dilute solutions has been studied sufficiently ([2]). In the present investigation this phenomenon was once again confirmed by direct electron-microscopic observations. In studying proteins that had passed through the membrane, it was possible to observe the entire sequence of complication of secondary structural formations, beginning with globules and bundles of chains and ending with the combination of bundles into fibrils and of fibrils into more complex structures. Of the numerous electron-microscopic photographs of secondary structural formations, the most characteristic are presented for gelatin in Fig. 2a (supramolecular formations from fibrils) and Fig. 2b (structure of individual fibrils), and for myosin in Fig. 3a (fibrils) and Fig. 3b (structure of fibrils). In Fig. 2a, gelatin fibrils 10 to 40,000 Å long are clearly distinguishable, without details of their fine structure. The thickness of such fibrils is approximately the same. Gelatin fibrils were also obtained that were very similar to collagen fibrils, with their characteristic transverse banding along the axis of the fibrils (Fig. 2b). It is difficult to evaluate the mode of packing of the bundles of gelatin chains in the fibrils; however, in the photograph shown, fibrils of spiral form are visible which, combining, form more complex structural systems.

The most consistent and complete picture of the ordering of structures was observed for myosin. Fig. 3a presents a fibrillar protein structure characteristic of myosin. At sufficient magnification, a clearly expressed discrete structure of the fine structural formations is detected (Fig. 3b). In the case of denatured egg albumin that had passed through the membrane, an analogous picture is observed. In Fig. 4, the complex structural formations arising after transfer of this product through the membrane are clearly distinguishable.

Thus, during electrodialysis, after destruction of protein structures and transfer of the elements of such structures through nonporous membranes, these elements again combine into the most diverse forms of secondary and more complex structural formations.

It is noteworthy that, regardless of the fibrillar or globular nature of a given protein, the synthesis of secondary structural formations, depending on definite

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experimental conditions (concentration, temperature, introduction of surface-active and similar substances, etc.), can lead to the appearance of both globules and fibrils, with subsequent complication of supramolecular structures. Apparently, the processes of destruction and restoration of structures in organisms of living nature are also connected with the role of semipermeable membranes and the action of the corresponding factors.

and components of the organism. Therefore, the study of such processes on the basis of their modeling is of undoubted interest for deepening our ideas about the essence of the entire diversity of life phenomena.

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